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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/786,988	01/23/1997	DANIEL P. LITTLE	24736-2001D	5922

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EXAMINER	
GAKH, YELENA G	

ART UNIT	PAPER NUMBER
1743	

DATE MAILED: 11/30/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	08/786,988	LITTLE ET AL.	
	Examiner	Art Unit	
	Yelena G. Gakh, Ph.D.	1743	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 October 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *108-146* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *108-146* is/are rejected.

7)☒ Claim(s) *119* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *101/17/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Amendment and Affidavit under 1.132 filed on 10/17/05 are acknowledged. Claims 108-146 are pending in the application.

Response to Amendment

2. Affidavit under 37 CFR 1.132 filed 10/17/05 is insufficient to overcome the rejection of claims 108-146 based upon the prior art as set forth in the last Office action because: the new prior art applied in the present Office action in response to the amendment provides the grounds for obviousness type rejection of the claims.

Claim Objections

3. Claim 119 is objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claim 119 recites 3-hydroxypicolinic acid, which is already recited in parent claim 108.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 144 and 145 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Since it may be assumed that the spots formed on the substrate are circular, it is not clear, which two dimensions 450μm x 450μm or 800μm x 800μm are meant in defining the spot size.

Moreover, the area of the spot would not be calculated as 450 x 450 μm^2, if 450μm is the spot diameter (D). It rather will be less than this product ($\pi/4D^2$).

Claim Rejections - 35 USC § 103

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

7. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. **Claims 108-146** are rejected under 35 U.S.C. 103(a) as being unpatentable over Nicola et al. (Rapid Commun. Mass Spectrom., 1995) in view of Li et al. (JACS, 1996, IDS), Hayes et al. (US 5,658,802, IDS) and Hancock et al. (US 5,716,825, IDS).

Nicola teaches "application of the fast-evaporation sample preparation method for improving quantification of angiotensin II by matrix-assisted laser desorption/ionization" with obtaining a substrate

for MALDI analysis comprising an array of matrix spots. Nicola emphasis increased reproducibility of mass spectra from spot to spot obtained by depositing an array of 2.5-10 μL droplets of the matrix on the MALDI substrate, drying the spots and applying ~ 1.0 μL of analyte/matrix solution on top of the dried matrix spots. While Nicola further indicates that "this resulted in greater crystal thickness compared to previously published results, which we consider essential for improvement in signal reproducibility (as previously reported, much less matrix was used for the matrix crystal layer, resulting in a much thinner layer)" (page 1166, left column), Li emphasizes that very reproducible spectra and results are obtained from repeated preparations (page 11663, right column) comprising depositing 0.9 μL of matrix solution forming "a very thin matrix layer" (page 11663, left column). Li also indicates that "the idea of microspot MALDI is to reduce the sample presentation surface with respect to the laser desorption site and ion acceptance volume in the mass spectrometer to improve the sample efficiency" (Li, page 11662, right column). Therefore, it would have been obvious for any person of ordinary skill in the art to further decrease volumes of MALDI matrix solutions deposited on the substrate compared to volumes used by Nicola for the reasons described by Li, i.e. "to reduce the sample presentation surface with respect to the laser desorption site and ion acceptance volume in the mass spectrometer".

While Nicola and Li do not specifically disclose disposing 0.2-20 nL of the matrix solution on the substrate, Hayes teaches "method and apparatus for making miniaturized diagnostic arrays" using electro-mechanical or piezoelectrical dispensers to place extremely small drops (10 pl to 1 nl) of fluid on substrates to form diagnostic arrays. Hayes indicates, "the invention thus provides a highly accurate, rapid and repeatable method of placing extremely small drops (10 pl to 1 nl) of fluid reagent on substrates to form diagnostic arrays. By using such small drops and accurately positioning them on the substrate, test strips can be formed which have a larger number of probes located within a smaller area than is achievable with prior methods" (col. 2, lines 49-55).

It would have been obvious for any person of ordinary skill in the art to modify Nicola's method by decreasing volumes of deposited matrix solution to 0.2-20 nL implying Hayes technique, because Li teaches microspot MALDI with highly reproducible results for picoliter amounts of the analyte when using very thin MALDI matrix spot formed on the probe wit an aim "to reduce the sample presentation surface with respect to the laser desorption site.

Nicola in view of Li and Hayes do not specifically disclose 3-hydroxypicolinic acid as a matrix or materials of the substrate other than steel.

Hancock discloses an integrated nucleic acid analysis system for MALDI-TOF MS, and describes in particular a thin film sample support, which is a substantially "planar manifold made of a non-conducting material that includes a microchannel and other necessary components of a miniturized sample preparation compartment, an interface to non-consumable parts, and an ionization surface for MALDI-TOF MS. Such a miniaturized device may be formed from a variety of materials (e. g., **silicon, glass, low cost polymers**) by techniques that are well known in the art (e.g., micromachining, chemical etching, laser ablation, and the like)" (col. 4,11. 34-44). Hancock further describes a process wherein analyte is embedded in a solid or crystalline "matrix" of light-absorbing molecules (e. g., **nicotinic, sinapinic, or 3-hydroxypicolinic acid**) (col. 6,11. 15-25). Hydrophobic and hydrophilic MALDI ionization surfaces, such as metals (**gold, copper, stainless steel**), glass, silica, nylon and other synthetic polymers, agarose and other carbohydrate polymers, and plastics are disclosed as surfaces for actively capturing analyte (col. 6,11. 38-44). Other capture regions are disclosed, such as **surface of a bead, particle** or planar support treated with a bifunctional cross-linking reagent. "According to the practice of the present invention, a capture region may be formed in any microstructure surface in the sample preparation compartment by linking an analyte binding partner directly to the surface, and on MALDI ionization surfaces integrated with the preparation compartment. Alternatively, a capture region may be formed on the surfaces of beads, which can be chemically attached to the surface of the support, or magnetically attached by using magnetically responsive beads and applying a magnetic field to anchor the beads to the desired region of the support. Magnetically responsive beads and particles are well-known in the art and are commercially available from, for example, Dynal. RTM., Inc. (Lake Success, N.Y.) and Bangs Laboratories, Inc. (Carmel, Ind.)" (col. 7, 11. 30-43).

It would have been obvious at the time the invention was made to a person having ordinary skill in the art to use 3-hydroxypicolinic acid as a matrix and the materials for the substrate described by Hancock in Nicola-Li-Hayes' methods, because Hancock discloses them as suitable materials for performing MALDI-MS analysis of biological materials, specifically DNA.

9. **Claims 108-146** are rejected under 35 U.S.C. 103(a) as being unpatentable over Vestal (US 5,498,545, IDS) in view of Vorm et al. (Anal. Chem., 1994), Hayes and Hancock et al. (US 5,716,825, IDS).

Vestal teaches an automated MALDI MS analysis for a plurality of samples, specifically DNA analytes, deposited as 100 nL droplets (col. 4, line 59) on a MALDI plate made of "stainless steel or

other suitable electrically conducting material" (col. 3, lines 57-58). The samples are prepared as mixtures of the analytes with MALDI matrix.

Vestal fails to teach depositing MALDI matrix without the analyte and allowing the spot to dry before depositing the analyte.

Vorm teaches a "fast evaporation" method of MALDI matrix deposition on a MALDI plate, comprising depositing ~ 0.5 μL drop of the matrix (ferulic acid, sinapic acid, etc.) on the MALDI stainless steel probe tip and allowing the spot to dry before applying a droplet of an analyte, indicating "improved resolution and very high sensitivity in MALDI TOF of matrix surfaces made by fast evaporation" (Title). "In the new procedure, matrix solution is applied to the probe tip of the mass spectrometer in a highly volatile solvent, e.g., acetone, to obtain very fast evaporation of the solvent. This leads to the formation of a dense, flat, and thin film presumably consisting of very small crystals of matrix. A small volume of analyte solution is then placed on top of the matrix surface, and the liquid is allowed to evaporate slowly. The only constraint on the analyte solution is that it must not completely re-dissolve the matrix surface, but only the outermost layer. We speculate that this layer is then doped with analyte molecules" (page 3282, left column). The solvents are acetone, acenotrile, methoanol, acetic acid and trifluoroacetic acid (page 3282). Moreover, Vorm specifically indicates, "the above procedure applies to large and flat probe tips. In systems which use small or curved probe tips slight alternations may be necessary to achieve homogeneous matrix surfaces. *The amount of matrix solution and its concentration can be used to adapt the procedure to the system used*" (page 3283, left column).

It would have been obvious for any person of ordinary skill in the art to modify Vestal's method of MALDI MS analysis by Vorm's method of depositing MALDI matrix as a droplet and allowing it to dry before depositing the analyte, because Vorm specifically indicates that it improves resolution and provides very high sensitivity in MALDI TOF analysis.

While Vestal and Vorm do not teach depositing drops of 0.2-20 nL, Hayes teaches "method and apparatus for making miniaturized diagnostic arrays" using electro-mechanical or piezoelectrical dispensers to place extremely small drops (10 pl to 1 nl) of fluid on substrates to form diagnostic arrays. Hayes indicates, "the invention thus provides a highly accurate, rapid and repeatable method of placing extremely small drops (10 pl to 1 nl) of fluid reagent on substrates to form diagnostic arrays. By using such small drops and accurately positioning them on the substrate, test strips can be formed which have a larger number of probes located within a smaller area than is achievable with prior methods" (col. 2,

lines 49-55). Different electro-mechanical dispensers comprising vesicles with chambers and transducers are disclosed in col. 2.

It would have been obvious for any person of ordinary skill in the art to modify Vestal-Vorm's MALDI MS analysis, including analysis of DNA by using Hayes' method of depositing very small droplets (less than 1 nL) of the matrix material because, as Hayes indicated, it allows creating a plurality of highly reproducible and volume-controlled spots, which is essential for obtaining reproducible MALDI spectra, the importance of which is well recognized in the art and because Vorm teaches that depositing small volumes of matrix solution in highly volatile solvents lead to formation of a dense, flat and thin film of very small crystals of matrix, which provide reproducible results for MALDI analysis.

Vestal in view of Vorm and Hayes do not specifically disclose 3-hydroxypicolinic acid as a matrix or materials of the substrate other than steel.

Hancock discloses an integrated nucleic acid analysis system for MALDI-TOF MS, and describes in particular a thin film sample support, which is a substantially "planar manifold made of a non-conducting material that includes a microchannel and other necessary components of a miniturized sample preparation compartment, an interface to non-consumable parts, and an ionization surface for MALDI-TOF MS. Such a miniaturized device may be formed from a variety of materials (e. g., **silicon, glass, low cost polymers**) by techniques that are well known in the art (e.g., micromachining, chemical etching, laser ablation, and the like)" (col. 4,11. 34-44). Hancock further describes a process wherein analyte is embedded in a solid or crystalline "matrix" of light-absorbing molecules (e. g., **nicotinic, sinapinic, or 3-hydroxypicolinic acid**) (col. 6,11. 15-25). Hydrophobic and hydrophilic MALDI ionization surfaces, such as metals (**gold, copper, stainless steel**), glass, silica, nylon and other synthetic polymers, agarose and other carbohydrate polymers, and plastics are disclosed as surfaces for actively capturing analyte (col. 6,11. 38-44). Other capture regions are disclosed, such as **surface of a bead, particle** or planar support treated with a bifunctional cross-linking reagent. "According to the practice of the present invention, a capture region may be formed in any microstructure surface in the sample preparation compartment by linking an analyte binding partner directly to the surface, and on MALDI ionization surfaces integrated with the preparation compartment. Alternatively, a capture region may be formed on the surfaces of beads, which can be chemically attached to the surface of the support, or magnetically attached by using magnetically responsive beads and applying a magnetic field to anchor the beads to the desired region of the support. Magnetically responsive beads and particles are

well-known in the art and are commercially available from, for example, Dynal. RTM., Inc. (Lake Success, N.Y.) and Bangs Laboratories, Inc. (Carmel, Ind.)" (col. 7, 11. 30-43).

It would have been obvious at the time the invention was made to a person having ordinary skill in the art to use 3-hydroxypicolinic acid as a matrix and the materials for the substrate described by Hancock in Vestal-Vorm-Hayes' methods, because Hancock discloses them as suitable materials for performing MALDI-MS analysis of biological materials, specifically DNA.

Response to Arguments

10. Applicant's arguments filed 10/17/05 have been fully considered but they are not fully persuasive.

Rejection of claims 144 and 145 35 U.S.C. 112, second paragraph. The Applicants' arguments are not quite clear. Spots are supposed to be planar; therefore, calling filling a well of an inverted pyramidal shape "spotting in well" is not quite apparent, especially when the spots are dried. If the spots have a square shape, because they are formed within the well having a square base, this needs to be clarified in the claims. Otherwise, since the spots are usually planar circles, two dimensions giving for spots are not clear.

The examiner understands that the Applicants' arguments related to the rejections over the prior art are based on Thomas Becker's Declaration. However, the examiner does not find any evidence in the prior art documents, which would teach away from using nano-scale volumes of MALDI matrix conventionally used specifically for DNA (i.e. 3-hydroxypicolinic acid) for obtaining reproducible MALDI results. Speaking in terminology of international applications used by the Applicants, the Applicants' methods and substrates are novel, but not inventive.

What the examiner fails to comprehend is that why it would not have been obvious for any routineer in the art to apply improved methods resulted from combination of Vestal, Vorm and Hayes, or Nicola and Hayes to MALDI analysis of DNA, which conventionally utilizes 3-hydroxypicolinic acid? The examiner indicated that the combination of the references teaches a method of obtaining MALDI substrates with improved reproducibility of the MALDI spots. It is not apparent to the examiner, why such improvement will not work for DNA analysis as it works for proteins? Moreover, Hancock

perfectly cures the deficiency of the combined teaching of Vestal, Vorm and Hayes, or Nicola and Hayes by providing disclosure of 3-hydroxypicolinic acid as a matrix for DNA MALDI analysis (which makes the statement of Thomas Becker that "the matrix 3-hydroxypicolinic acid (3-HPA) is useful for mass spectrometric analysis of nucleic acid molecules, *and is not discussed in the document cited in the Office acid*" somehow puzzling).

Regarding subparagraph 3 of the Declaration. The Applicant is absolutely correct in that Nicola does not teach depositing nanoliter amounts of the matrix. However, Li indicates that depositing picoliter amounts of the analyte on a very thin layer of a matrix gives highly reproducible results. Nicola's disclosure is explicit regarding improved results when the matrix is applied first, dried, and the analyte is applied afterward. Again, there is no assumption expressed by Nicola or Li, that this technique will not work for 3-HPA and DNA MALDI. It is not a big surprise that the spot obtained from depositing nanoliter amounts of a solution is several times smaller than the spot obtained from depositing microliter amounts of the solution. The opposite would be quite unexpected.

In conclusion, the examiner would like to note that the specification was silent regarding unexpected results obtained specifically for MALDI analysis of DNA using nanoliter amounts of 3-HPA. The explanation for applying smaller amounts of solutions was quite different: "it is understood that the above methods provide processes that allow for rapidly dispensing definite and controlled volumes of analyte material. In particular these processes allow for dispensing sub to low nanoliter volumes of fluid. These low volume deposition techniques generate sample arrays well suited for analysis by mass spectrometer. For example, the low volumes yield reproducibility of spot characteristics, such as evaporation rates and reduced dependence on atmospheric conditions such as ambient temperature and light" (page 19).

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Yelena G. Gakh, Ph.D. whose telephone number is (571) 272-1257. The examiner can normally be reached on 9:30 am - 6:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill A. Warden can be reached on (571) 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

11/28/05

Yelena Gakh

YELENA GAKH
PRIMARY EXAMINER